



Improving Myocardial Protection: The Key Variables which Affect Troponin Release after CABG.

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Highlights

Background

Myocardial cell ischaemic injury during cardiopulmonary bypass and aortic crossclamp remains a key limiting factor to patient outcomes in coronary artery bypass grafting. Troponin I has been shown to be an effective indicator of myocardial ischaemic injury and achieves peak levels early post-operatively.

Methods

All consenting CABG patients from one centre, during a one year period, were recruited. All surgeries were performed using identical techniques besides the cardioplegia volume and number of doses. Troponin I levels were checked regularly post-operatively until a peak troponin I level was ascertained. All the patient demographics, crossclamp times, bypass times and cardioplegia dosing were analysed using multiple combinations of statistical tools.

Results

172 patients were included in the study and cardiopulmonary bypass (CPB) time was found to be significant as a single variable ($p=0.033$). The combination of CPB time and ischaemia time ($p=0.002$) and the combination of CPB time and multidose cardioplegia ($p=0.009$), were both found to significantly affect peak troponin I levels. Another analysis was performed on the volume of cardioplegia used. While this was not significant as an individual variable it did become significant when combined with ischaemia time at a threshold total cardioplegia volume of 750mls ($p=0.026$).

Conclusions

The conclusion therefore is that using over 750mls of cardioplegia in multiple doses will safely protect against an ischaemia time of up to 62min. However there is no protection against the CPB time, which proved to have the most impact on myocardial cell damage in our practice.

Keywords: Cardioplegia; Myocardial protection; Ischaemic cell injury; Cardiopulmonary bypass; Coronary artery bypass graft.

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Introduction

Coronary artery bypass grafting (CABG), via median sternotomy is the most common cardiac operation [1]. This operation may be associated with several complications and one of the most significant is myocardial damage. The usual technique involves the institution of cardiopulmonary bypass (CPB) and inducing cardiac arrest in diastole using potassium rich cardioplegia with cross clamping of the aorta and a consequent ischaemic period to the heart. The myocardial damage occurring during

this ischaemic period is attenuated by inducing cardiac arrest in diastole and by lowering myocardial temperature [2,3]. Various myocardial management strategies exist and each method has its advantages and limitations.

This study was performed using one method of myocardial protection to assess the effect of several variables on myocardial protection. The possible variables that could influence

myocardial protection were the patient's demographics, the total volume of cardioplegia given and the number of divided doses it was given in, the aortic cross clamp (ischaemic) time and the CPB time.

An effective and sensitive way of monitoring myocardial cell damage is through measurement of early post-operative myocardial enzyme release [4,5]. Troponin I was identified as the most widely used marker in the literature and was readily available in the hospital during this study [6]. Most studies indicated that the peak value of post-operative troponin I was the most significant indicator of myocardial injury, with levels reaching almost 20 times the normal reference [7]. In most cases troponin I peaked within 18 hours of surgery and decreased steadily thereafter [8].

Methods

All patients undergoing elective CABG over a one-year period (January-December 2011) were recruited prospectively. Written consent was obtained from all patients and the study was endorsed by the local research council. The surgeries were performed by the same 5 surgeons using identical techniques. Median sternotomy was performed followed by harvest of the left internal thoracic artery (LITA) and institution of full aorto-atrial cardiopulmonary bypass. Cold blood cardioplegia using Martindale Pharma® cardioplegia concentrate (20mls diluted with 180mls of saline) and mixed with 800mls of blood was infused antegradely. The LITA was grafted on the left anterior descending artery. Long saphenous vein segments were harvested using an open technique and were used to bypass the other coronary arteries. After the distal anastomoses were completed the cross clamp was removed allowing cardiac activity to resume and the proximal anastomoses were sutured to punched holes in the aorta. The LITA perfused the heart for 2 minutes prior to the release of the aortic cross clamp and no special reperfusion was used in any of the cases. Patients suffering from a postoperative myocardial infarction, diagnosed on clinical, electrocardiographic and echo- cardiographic features were excluded from analysis.

Troponin I levels were taken at 2, 6, 12, 24 and 48 hours post-operatively. Sample collection was stopped if the results showed that the troponin levels began to decrease. The highest value was taken as the peak troponin level. All data including demographics and pre-operative criteria such as logistic Euroscore, haematocrit, left main stem lesions, and renal function were recorded. Intra-operative values such as cardioplegia volume, timing of each of cardioplegia infusion, duration of cardiac ischaemia and cardiopulmonary bypass times, presence and nature of arrhythmias, intra-operative haematocrit, acid-base status and arterial and graft size and quality were documented. Immediately post-operative inotrope requirements and use of intra-aortic balloon pumps were documented.

Statistical Analysis

All the data collected was analysed with respect to the peak troponin I levels using SPSS v.21 (IBM) by a professional statistician. A logarithmic transformation to troponin I values was carried out to reduce the data skewness. The normality assumption was then checked using the Kolmogorov Smirnov test. The individual criteria were compared to the peak troponin I levels using the one-way ANOVA test if the variables were

categorical or a bivariate Pearson two-tailed correlation test if the variables were metric. A 0.05 level of significance was adopted for all tests.

The goal of this study is to assess the collective impact of the individual criteria on troponin I. This was carried out by fitting a General Linear Model (GLM) assuming a Normal distribution and an identity link function. This model related the log-transformed troponin I to the individual criteria that were identified to be significant or almost significant in univariate analysis.

Results

The sample comprised 174 patients, two of whom were removed because of incomplete information, totalling 172 patients (83% males and 17% females). The average age was 64.4 years with 27% of patients being over 70 years. 52% of patients underwent a CABG x3, while 34% underwent CABG x2. Only 4.7% and 8.7% received 1 or 4 grafts respectively.

The peak post-operative troponin I level ranged from 0.7 to 180 (normal range 0-0.29mg/ml), with a mean of 13.5 and a median of 5.9. 13.5% of patients had a troponin above 20, therefore the majority where within 0.7 and 20.

The bypass time distribution, ranging from 25 till 116 minutes, is marginally right skewed and the average bypass time for 171 patients was 65 minutes after eliminating one patient due to missing data.

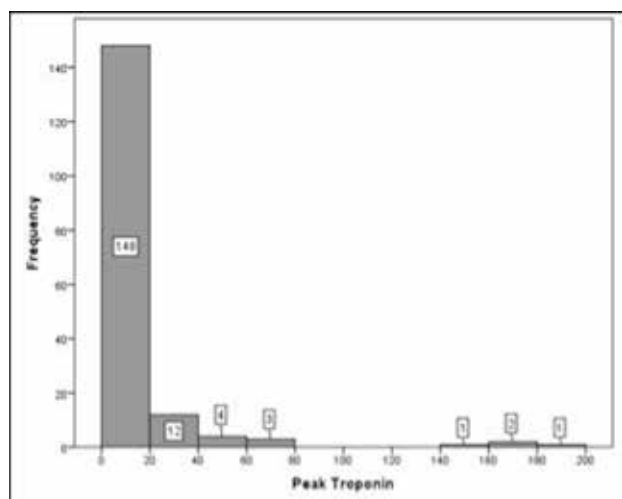
The Kolmogorov-Smirnov test was first used to assess the normality assumption of the peak troponin I level distribution. The null hypothesis specifies that the distribution is normal and is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that the distribution is skewed and would be accepted if the p-value is less than the 0.05 criterion.

It is clear from the histogram and Kolmogorov-Smirnov test that the peak troponin I level distribution is considerably right skewed and does not satisfy the normality assumption (graph 1).

The same test was used to assess the normality assumption of log-transformed peak troponin distribution. The histogram (graph 2) shows that the Log Peak Troponin I distribution is marginally right skewed; however the Kolmogorov Smirnov test indicates that the normality assumption is satisfied since the p-value ($p=0.136$) exceeds the 0.05 criterion.

Log Peak Troponin I levels was related to each individual criterion using either Pearson correlation, if the criterion had a metric scale, or the One-Way ANOVA test if the criterion had a categorical scale. Table 1 displays the p-value of each univariate analysis.

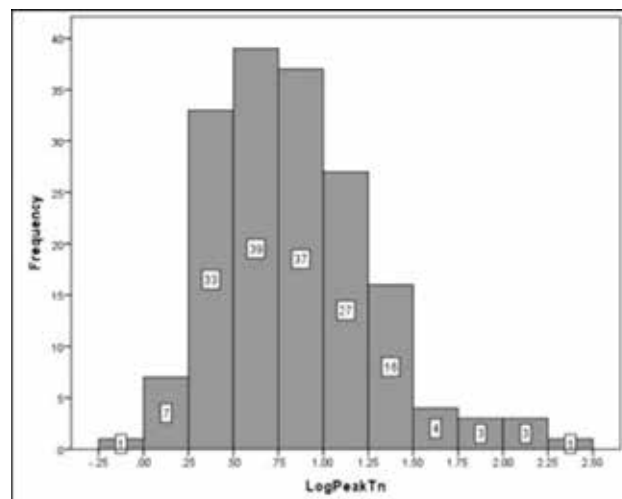
The scatter plot (graph 3) displays the linear relationship between ischaemia time, bypass time and the Log Peak Troponin. All relationships are positive, which implies that an increase in one variable results in an increase in the other two variables. The relationship between ischaemia time and bypass time is the stronger one, while the relationship between log peak troponin and ischaemic time is the weaker one.



Graph 1. Peak Troponin distribution

Kolmogorov-Smirnov test to assess normality of peak troponin distribution

One-Sample Kolmogorov-Smirnov Test	
	Peak Troponin
Kolmogorov-Smirnov Z	4.128
P-value (2-tailed)	0.000



Graph 2. Log-Peak Troponin distribution

Kolmogorov-Smirnov test to assess normality of log-peak troponin distribution

One-Sample Kolmogorov-Smirnov Test	
	Peak Troponin
Kolmogorov-Smirnov Z	1.159
P-value (2-tailed)	0.136

The log-transformed peak troponin I levels was analysed further using general linear models with log peak troponin I as the dependent variable, and bypass time, ischaemia time, number of CP doses and total CP volume as the predictors. The pairwise interaction effect between the predictors was also analysed. In some of the models the number of CP doses was categorised as (no dose, multiple doses) and total CP volume was categorised as (below 750, above 750).

GLMs were also used to assess the impact of any two strong predictors and their interaction effect on log peak troponin I levels. Significance was noted when analyzing cardiac ischaemia time. The significant interactions were with ventricular fibrillation (VF) on removing the cross clamp ($p=0.017$) and with the total volume of cardioplegia (CP).

Graph 4 shows the relationship between log peak troponin, bypass time and a grouped version of the number of CP doses. Tests of between-subject effects show that both main effects and their interaction effects are significant since their p-values are less than the 0.05 level of significance. Graph 4 displays that log peak troponin increases with an increase in bypass time; however this increase is more conspicuous for patients taking one dose. For patients given one CP dose, the log peak troponin is larger in a long bypass durations and smaller in short bypass durations, compared to patients given multiple CP doses.

Log peak troponin was examined further by clustering the patients in two cardioplegia volume groups; those receiving more than a certain CP dose and those receiving less. This threshold CP dose was incremented in steps of 50 ml and each time analyzed using

GLM. Models using threshold CP doses of 700 ml or less yielded no significant results; however, models using threshold CP doses of 750 ml or more yielded interesting results. Graph 5 displays the relationship between log peak troponin, ischaemia time and a grouped version of CP volume. It shows that log peak troponin increases with an increase in ischaemia time; however this increase is more conspicuous for patients taking less than 750 ml. For patients given less than 750 ml CP dose, the log peak troponin is larger in a long ischaemia times and smaller in short ischaemia times, compared to patients given more than 750 ml CP dose. Tests of between-subject effects show that none of the effects are significant at the 0.05 level of significance, indicating that these relationships may be attributed to chance.

Graph 6 displays the relationship between log peak troponin, CP volume and a grouped version of the number of CP doses. It shows that log peak troponin increases marginally with an increase in total CP volume when one CP dose is provided; however, log peak troponin decreases marginally with an increase in total CP volume when multiple CP doses are provided. Tests of between-subject effects show that none of the effects are significant at the 0.05 level of significance, indicating that these relationships may be attributed to chance.

Graph 7 displays the relationship between log peak troponin, ischaemia time and a grouped version of the number of CP doses. It shows that log peak troponin increases with an increase in ischaemia time; however this increase is slightly more conspicuous for patients taking one CP dose. Tests of between-subject effects show that none of the effects are significant at the 0.05 level, indicating that these relationships may be attributed to chance.

Table 1. P-values of each univariate analysis

Predictor	P Value	Predictor	P Value
Logistic Euroscore	0.062	pH	0.953
Age	0.056	Na	0.772
Sex	0.431	Cl	0.483
Weight	0.230	Ca	0.224
BSA	0.163	Gluc	0.229
DM	0.447	Creatinine	0.014
HT	0.964	Dopamine	0.944
Unstable Angina	0.565	Adrenaline	0.001
Vol 1	0.726	Noradrenaline	0.001
Ischaemia 1	0.889	Milrinone	0.958
Vol 2	0.389	IABP	0.017
Ischaemia 2	0.683	EDV	0.085
Vol 3	0.243	ESV	0.952
Ischaemia 3	0.416	SV	0.864
Vol 4	0.326	LMS	0.566
Ischaemia 4	0.135	Diffuse Disease	0.426
Total CP Vol	0.956	Statins	0.545
Ischaemia Time	0.097	Vastarel	0.107
Ejection fraction	0.090	VF during plegia and shock	0.615
Bypass time	0.033	VF after removing cross clamp	0.907
CP Potassium	0.408	Multidose CP	0.092
CP O2	0.636	Total CP vol above or below 750	0.932
Hct	0.070		

P-values marked in yellow are less than the 0.05 level of significance, while p-values marked in green range from 0.05 to 0.1. Ischaemia 1,2,3 and 4 refer to the time in-between doses of cardioplegia.

Discussion

The number of patients included was of adequate size with a good demographic and geographical representation. Around a quarter of patients were over 70-years-old, logistic Euroscores varied from 0.88 to 34.5 (mean: 2.3, median: 1.68) and half were below 2. The duration of cardiac ischaemia varied from 11 to 62 minutes (mean: 34.9 minutes), however ejection fraction was predominantly good with only 9% being moderate and no cases of poor ventricular function. This therefore means that the conclusions drawn from this study may not be applicable to cases with very low ejection fractions. The contents of the cardioplegia solution was identical throughout all the cases and the troponin I tests were analyzed by the same laboratory using the Immulite 2000® kits throughout the entire study.

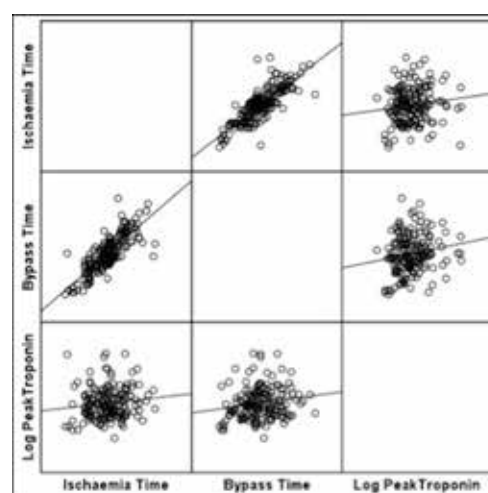
The first set of results came from analyzing one variable against peak troponin I levels (table 1). There were several factors showing immediate significance such as the patients pre-operative creatinine, the administration of adrenaline or noradrenaline post-operatively and the use of an intra-aortic balloon pump. It is generally acknowledged that the more unstable patients will sustain more myocardial injury and that

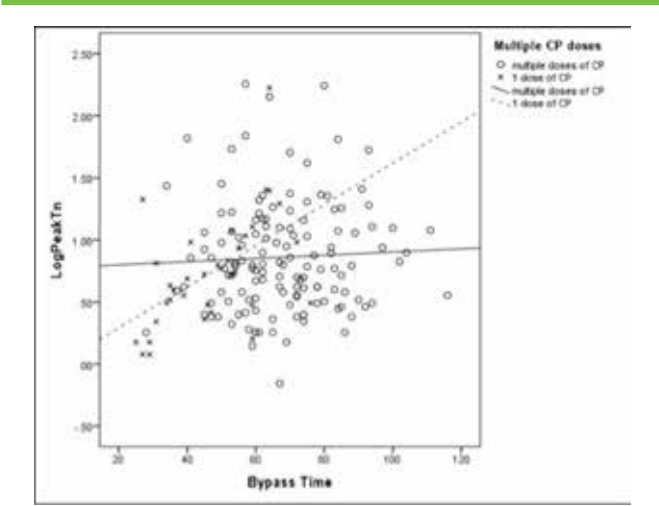
the use of inotropes in themselves results in more myocardial enzyme release [9,10].

This first set of results display an interesting relationship, where it was shown that the bypass duration has a significant effect on the peak troponin I levels and hence on myocardial injury, however the relationships of peak troponin I levels with cardiac ischaemia times and cardioplegia volumes were not significant. This indicates that cardioplegia protects against myocardial ischaemic damage and therefore as single variables they will not have a direct effect on the peak troponin I levels. This, however, needs to be interpreted within the context of the time range in this study and with a maximum ischaemia time of 62 minutes, one can assume that this may not be the case in longer procedures. The conclusion thus far can be made that in coronary artery graft surgery of around one hour duration with standard myocardial protective techniques the ischaemia time is being well protected against and yet the time spent on bypass without myocardial protection causes significant myocardial injury.

The second set of results were obtained using a GLM to assess the impact of two variables and their interaction effect on peak troponin I level. Since CPB time was significant independently from other factors, the significance was not altered by any other factor besides the ischaemia time and the delivery of cardioplegia in multiple doses as opposed to one single dose. As shown in graph 3 the CPB and ischaemia time curves are positively and significantly related. This is in-keeping with current knowledge as longer procedures will include lengthening both times, however the previous observation shows they are not independently linked. The conclusion therefore is that while bypass time is itself significant on the peak troponin, the ischaemia time is not. The two-way ANOVA models with interaction, described in the results section, only identify bypass time as a significant predictor of peak troponin. The recommendation is that, within normal limits (62 minutes in this study), ischaemic injury is well suppressed with adequate cardioplegia, however prolongation of CPB is associated with more troponin release indicating an insult to the myocardium.

These findings may suggest that CPB per se causes myocardial damage independently of cross clamp induced myocardial ischaemia. Also the inference might be made that less


Graph 3. Scatter plot showing relationships between Ischaemia time, Bypass time and Log Peak Troponin



Graph 4. Relationship between log peak troponin, bypass time and CP doses

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Table 4 Tests of Between-Subjects Effects
(Dependent variable – Log PeakTroponin)

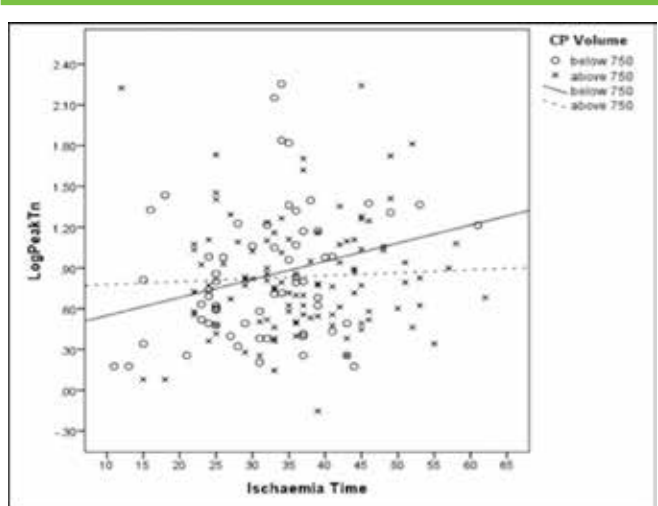
Source	Sum of Squares	df	Mean Square	F	P-value
Intercept	1.070	1	1.070	5.751	0.018
ByPassTime	1.779	1	1.779	9.564	0.002
MultidoseCP	1.317	1	1.317	7.078	0.009
MultidoseCP * ByPassTime	1.303	1	1.303	7.003	0.009
Error	30.69	165	0.186		

Graph 4 shows the relationship between log peak troponin, bypass time and a grouped version of the number of CP doses. Tests of between-subject effects show that both main effects and their interaction effects are significant since their p-values are less than the 0.05 level of significance. Graph 4 displays that log peak troponin increases with an increase in bypass time; however this increase is more conspicuous for patients taking one dose. For patients given one CP dose, the log peak troponin is larger in a long bypass durations and smaller in short bypass durations, compared to patients given multiple CP doses.

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myocardial damage occurs if the cardioplegia protection of the heart with an aortic cross clamp is increased with consequent reduction of beating heart CPB unprotected by cardioplegia. One such scenario is the performance of aorto-saphenous vein anastomoses (proximal anastomoses) with the aortic cross clamp in situ hence delaying myocardial activity during CPB. This may be the reason why the patients receiving multidose cardioplegia had some protection against a longer bypass time (graph 4). It is possible that the effect of cardioplegia continued into the proximal anastomosis and the delayed onset of myocardial activity allowed for better protection.

The concept of adequate cardioplegia mentioned earlier was also assessed using this data. The total amount of cardioplegia solution given and its administration in multiple doses rather than one single dose was linked to longer CPB and cardiac ischaemia times. The critical volume of cardioplegia below which there was a statistically significantly larger release of troponin was 750mls. Hence, a total of 750mls or more of



Graph 5. Relationship between log peak troponin, ischaemia time and CP volume

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Table 5. Tests of Between-Subjects Effects
(Dependent variable – Log PeakTroponin)

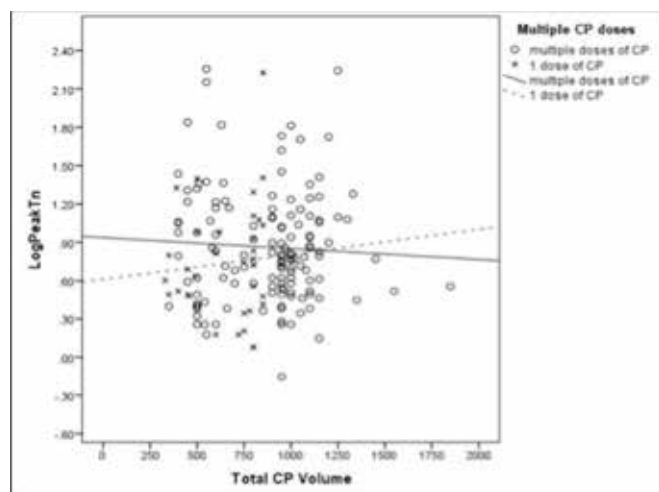
Source	Sum of Squares	df	Mean Square	F	P-value
Intercept	4.068	1	4.068	20.888	0.000
IschaemiaTime	0.669	1	0.669	3.431	0.066
CatCPVol	0.342	1	0.342	1.757	0.187
CatCPVol * IschaemiaTime	0.451	1	0.451	2.316	0.130
Error	31.94	164	0.195		

Graph 5 displays the relationship between log peak troponin, ischaemia time and a grouped version of CP volume. It shows that log peak troponin increases with an increase in ischaemia time; however this increase is more conspicuous for patients taking less than 750 ml. For patients given less than 750 ml CP dose, the log peak troponin is larger in a long ischaemia times and smaller in short ischaemia times, compared to patients given more than 750 ml CP dose. Tests of between-subject effects show that none of the effects are significant at the 0.05 level of significance, indicating that these relationships may be attributed to chance.

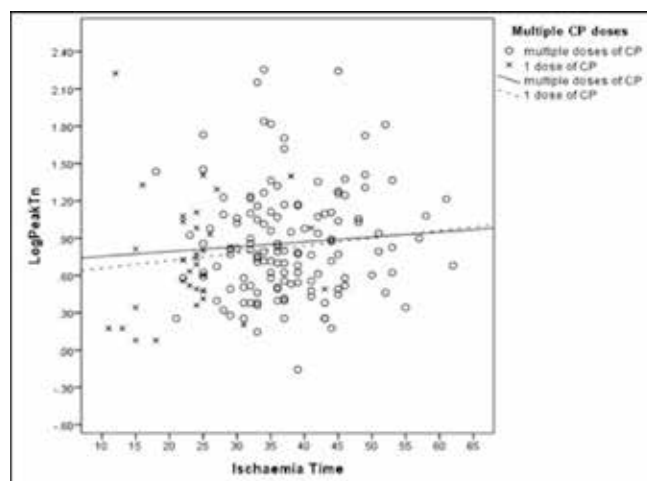
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cardioplegia given in multiple doses as required offer the best protection for moderate to good ejection fraction patients within ischaemic times of up to 62 minutes and a CPB time of up to 116 minutes. As shown in graph 5 the ischaemia time will only raise the peak troponin if too little cardioplegia is given. Independently, ischaemia time and total cardioplegia volume did not show significant effect on peak troponin I levels but the combined effect attained significance. The statistical significance of this combination increases as the total volume of cardioplegia administered increases. The timing of these multiple doses did not have any significant effect on the peak troponin when identified as individual variables in table 1 (ischaemia 1,2,3 and 4).

There are multiple reasons why the time spent on CPB causes greater myocardial cell damage. An important factor is the haemodynamic changes in coronary blood flow during the non-pulsatile flow of CPB. The collapsed heart causes increased subendocardial vascular resistance and when this is coupled with a narrow pulse pressure and a reduced mean arterial



Graph 6. Relationship between log peak troponin, CP volume and CP doses



Graph 7. Relationship between log peak troponin, ischaemia time and CP doses

Table 6 Tests of Between-Subjects Effects (Dependent variable – Log PeakTroponin)

Source	Sum of Squares	df	Mean Square	F	P-value
Intercept	4.645	1	4.645	23.613	0.000
TotalCPVol	0.012	1	0.012	0.061	0.805
MultidoseCP	0.207	1	0.207	1.051	0.307
MultidoseCP * TotalCPVol	0.079	1	0.079	0.399	0.528
Error	32.46	165	0.197		

Graph 6 displays the relationship between log peak troponin, CP volume and a grouped version of the number of CP doses. It shows that log peak troponin increases marginally with an increase in total CP volume when one CP dose is provided; however, log peak troponin decreases marginally with an increase in total CP volume when multiple CP doses are provided. Tests of between-subject effects show that none of the effects are significant at the 0.05 level of significance, indicating that these relationships may be attributed to chance.

pressure the result is poorer perfusion of the subendocardial layer [11]. The subendocardial layer is very vulnerable to ischaemia, partly due to its higher oxygen requirements [12]. Furthermore the relatively empty left ventricle reduces the direct contact of intraventricular oxygenated blood to the subendocardial layers [13]. Also, the generalised inflammatory response induced by CPB, causing platelet aggregation and endothelial activation may induce microscopic occlusions of tiny myocardial capillaries [14]. When considering that all the patients in this study suffer from ischaemic heart disease it is plausible that they have little reserve to tolerate any of the above changes and therefore suffer ischaemia during the CPB which may be of greater significance than the aortic cross-clamp time.

Limitations

The best method available during this study to identify myocardial cell damage was a serum troponin level. This investigation is significantly altered by postoperative ischaemia and infarction. Therefore the intraoperative damage may not have been the cause of the raised troponin values in some patients. Every effort was made to exclude patients with any sign of post-operative

Table 7 Tests of Between-Subjects Effects (Dependent variable – Log PeakTroponin)

Source	Sum of Squares	df	Mean Square	F	P-value
Intercept	3.388	1	3.388	17.170	0.000
IschaemiaTime	0.145	1	0.145	0.736	0.392
MultidoseCP	0.026	1	0.026	0.132	0.716
MultidoseCP * IschaemiaTime	0.007	1	0.007	0.033	0.855
Error	32.36	164	0.197		

Graph 7 displays the relationship between log peak troponin, ischaemia time and a grouped version of the number of CP doses. It shows that log peak troponin increases with an increase in ischaemia time; however this increase is slightly more conspicuous for patients taking one CP dose. Tests of between-subject effects show that none of the effects are significant at the 0.05 level, indicating that these relationships may be attributed to chance.

ischaemic events that would alter their troponin level. However once a patient was recruited into the study no selection bias was permitted and even those with seemingly excessive troponin rises were included.

Another limitation is the absence of patients with poor ventricular function and the small number of patients with moderate ventricular function. This was not an intentional selection bias but was purely coincidental. In a small unit such as this one the majority of routine CABG cases have a good ejection fraction. This limits the study in that the conclusions can only be applied to patients with moderate to good ejection fractions and must not be applied to weaker hearts which may require more insense myocardial protection strategies.

Conclusion

In patients with moderate or good ventricular function, myocardial protection using 750 mls or more of cold blood cardioplegia is sufficient to prevent the ischaemic time from significantly affecting the post-operative peak troponin levels. The same can not be said for the bypass time which has a direct linear effect on post-operative troponin levels and therefore must be considered as a vulnerability in standard myocardial protection techniques.



The ischaemia time is shown to effect post-operative troponin when less than 750mls of cardioplegia is given. This confirms that adequate myocardial protection requires a total cardioplegia volume of 750 mls or more. The data also shows that this is ideally given in multiple doses as required but the specific timing of these doses or the number of separate cardioplegia injections were not significant.

Declarations of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors state that they abide by the "Requirements for Ethical Publishing in Biomedical Journals"(15).

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